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TREATMENT OF GRAM-NEGATIVE BACTEREMIA AND SEPTIC SHOCK WITH HA-1A HUMAN MONOCLONAL ANTIBODY AGAINST ENDOTOXIN

A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract *Background.* HA-1A is a human monoclonal IgM antibody that binds specifically to the lipid A domain of endotoxin and prevents death in laboratory animals with gram-negative bacteremia and endotoxemia.

Methods. To evaluate the efficacy and safety of HA-1A, we conducted a randomized, double-blind trial in patients with sepsis and a presumed diagnosis of gram-negative infection. The patients received either a single 100-mg intravenous dose of HA-1A (in 3.5 g of albumin) or placebo (3.5 g of albumin). Other interventions, including the administration of antibiotics and fluids, were not affected by the study protocol.

Results. Of 543 patients with sepsis who were treated, 200 (37 percent) had gram-negative bacteremia as proved by blood culture. For the patients with gram-negative bacteremia followed to death or day 28, there were 45 deaths among the 92 recipients of placebo (49 percent) and 32 deaths among the 105 recipients of HA-1A (30 percent; P = 0.014). For the patients with gram-negative bacteremia and shock at entry, there were 27 deaths among the 47 recipients of placebo (57 percent) and 18 deaths among the 54 recipients of HA-1A (33 percent; P = 0.017). Analyses that stratified according to the severity of illness at entry showed improved survival with HA-1A treatment in both severely ill and less severely ill patients. Of the 196 patients with gram-negative bacteremia who were followed to hospital discharge or death, 45 of the 93 given placebo (48 percent) were discharged alive, as compared with 65 of the 103 treated with HA-1A (63 percent; P = 0.038). No benefit of treatment with HA-1A was demonstrated in the 343 patients with sepsis who did not prove to have gram-negative bacteremia. For all 543 patients with sepsis who were treated, the mortality rate was 43 percent among the recipients of placebo and 39 percent among those given HA-1A (P = 0.24). All patients tolerated HA-1A well, and no anti–HA-1A antibodies were detected.

Conclusions. HA-1A is safe and effective for the treatment of patients with sepsis and gram-negative bacteremia. (N Engl J Med 1991; 324:429-36.)

SEPTICEMIA is a leading cause of morbidity and mortality among hospitalized patients. There are approximately 400,000 cases each year in the United States, and the incidence continues to increase. Gram-negative bacteremia occurs in about 30 percent of patients with septicemia.[2] Despite the use of potent antibiotics and intensive supportive care, the mortality among patients with sepsis and gram-negative bacteremia remains high. It varies from 20 to 60 percent, depending on the specific population.[2-4]

Bacteremia and septic shock are associated with the release of endotoxin into the circulation.[5,6] Endotoxin is the lipopolysaccharide component of the cell walls of gram-negative bacteria that triggers many of the adverse systemic reactions and serious sequelae in patients with sepsis and gram-negative bacteremia. Im-

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Ref 2148

munotherapy with human polyclonal antiserum or plasma directed against endotoxin core determinants has been shown in trials to reduce mortality in patients with gram-negative bacteremia[7] and to protect high-risk surgical patients from septic shock.[8] The antiserum used in those trials was developed by immunizing volunteers with heat-inactivated cells of the J5 mutant of *Escherichia coli* 0111:B4, which induce an immune response to the core region of endotoxin. The region is shared among gram-negative bacterial species and contains lipid A, thought to be the toxic moiety of endotoxin.

Polyclonal J5 antiserum is not commercially available for several reasons. Vaccinating serum donors with J5 vaccine is associated with mild toxicity; there is no booster response, so a person can donate only once; the antibody content of antiserum preparations varies; and there is the potential for transmitting infection with pooled human blood. The use of monoclonal-antibody techniques circumvents these problems and allows the production of large quantities of antibody of known isotype and epitope specificity. Furthermore, a human monoclonal antibody offers the potential advantage of better effector function than antibodies from other species.

HA-1A (Centoxin) is a human monoclonal IgM antibody that binds to the lipid A domain of endotoxin and is produced by the stable heteromyeloma cell line A6(H4C5) developed by Teng, Kaplan, and Braude.[9] The same heat-inactivated *E. coli* J5 vaccine that induced polyclonal J5 antiserum was used in the development of this cell line. HA-1A has been shown to bind specifically to many endotoxins and to a broad range of clinical isolates of gram-negative bacteria. In various animal models of gram-negative bacteremia and endotoxemia, the administration of HA-1A after challenge prevents the development of the dermal Shwartzman reaction and death.[9,10]

To determine the efficacy of HA-1A in reducing the mortality associated with gram-negative bacteremia, we conducted a prospective, multicenter, randomized, double-blind, placebo-controlled clinical trial in patients with the sepsis syndrome and a presumptive diagnosis of gram-negative infection.

Methods

The efficacy and safety of HA-1A were assessed in a double-blind fashion and analyzed according to a prospectively developed plan that used definitions adopted before the treatment-allocation code was broken. The analysis focused on the patients with gram-negative bacteremia because this group had proved gram-negative infection with a high likelihood of endotoxemia,[5,6] but all treated patients were analyzed.

An independent coordinating center (Maryland Medical Research Institute, Baltimore) was responsible for creating a treatment-allocation code for each site, labeling vials, monitoring compliance with the blinding procedures, auditing the data for consistency and accuracy, and conducting the interim analyses. The coordinating center appointed a Safety and Efficacy Monitoring Committee to oversee the trial, which was undertaken at 24 academic medical centers in the United States, Canada, and Europe. The protocol was approved by the institutional review board at each hospital, and informed consent was obtained from all participants.

Patient Selection

Patients with sepsis and suspected gram-negative infection were enrolled in this clinical trial by their physicians and randomly assigned to receive HA-1A or placebo. The criteria for a diagnosis of sepsis were as follows: fever or hypothermia (temperature >38.3°C [101°F] or <35.6°C [96°F]); tachycardia (>90 beats per minute in the absence of beta-blockade) and tachypnea (respiratory rate >20 breaths per minute or the requirement of mechanical ventilation); and either hypotension (systolic blood pressure ≤90 mm Hg or a sustained drop in systolic pressure ≥40 mm Hg in the presence of an adequate fluid challenge and the absence of antihypertensive agents) or two of the following six signs of systemic toxicity or peripheral hypoperfusion: unexplained metabolic acidosis (pH

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≤7.3, base deficit of >5 mmol per liter, or an elevated plasma lactate level); arterial hypoxemia (partial pressure of oxygen ≤75 mm Hg or ratio of the partial pressure of oxygen to the fraction of inspired oxygen <250); acute renal failure (urinary output of less than 0.5 ml per kilogram of body weight per hour); elevated prothrombin or partial-thromboplastin time or reduction of the platelet count to less than half the base-line value or less than 100,000 platelets per cubic millimeter; sudden decrease in mental acuity; and cardiac index of more than 4 liters per minute per square meter of body-surface area with systemic vascular resistance of less than 800 dyn · sec · cm^{-5}.

Patients were not eligible for the trial if they were less than 18 years of age; if pregnancy was suspected; if their condition was clearly irreversible, with a rapidly fatal course; if they had undergone organ transplantation; if uncontrolled hemorrhage, cardiogenic shock, or burn injury was the primary acute underlying condition, or if they had been given any monoclonal antibodies or intravenous immunoglobulins (except fresh-frozen plasma) within 21 days.

Treatment

HA-1A is produced by continuous-perfusion cell culture and is purified from the supernatant fluid by a series of steps involving selective precipitation and column chromatography. The cell line that produces HA-1A has been tested extensively and has been shown to be free of human viruses. Furthermore, the purification process for HA-1A includes specific viral-inactivation procedures, and tests are performed to confirm the absence of viruses. None of the lots contained detectable endotoxin in an assay with a sensitivity of 3 pg per milliliter.

Patients enrolled in the trial were randomly assigned to receive either 100 mg of HA-1A diluted with 3.5 g of human serum albumin or a placebo consisting of 3.5 g of human serum albumin. The contents of each vial were diluted to a final volume of 50 ml and given in a single intravenous infusion over a period of 15 to 20 minutes.

Decisions regarding the use of antibiotics, intravenous fluids, cardiovascular and respiratory support, and surgical intervention were made by each patient's attending physicians and were not dictated by the study protocol.

Evaluation of the Patients

Patients were followed for 28 days or until death. An APACHE II score, a severity-of-illness index,[11] was calculated at entry. Samples of blood and all other suspected foci of infection were obtained for culture within the 24 hours before enrollment. Vital signs (blood pressure, temperature, heart rate, and respiratory rate) were recorded frequently during the first 12 hours after the infusion of HA-1A or placebo and then on days 1, 2, 3, 5, 7, 14, and 28. Routine hematologic and clinical chemistry tests were obtained before infusion, 12 hours after infusion, and daily thereafter until the values were normal.

Serum for the determination of anti–HA-1A antibody levels was obtained before and approximately 28 days after infusion. Serum was assayed for anti–HA-1A antibodies at the core laboratory with a double-antigen radiometric assay similar to that described previously.[12] The sensitivity of the assay was 0.35 μg of antibody per 0.1 ml of serum.

Definitions and Criteria

To minimize site-to-site variation in the classification of patients, a four-member Clinical Evaluation Committee was organized, as dictated by the study protocol. The committee established the definitions used in the trial and classified each case with respect to underlying disease, culture status, primary site of infection, causative organism, adequacy of antibiotic therapy, and adequacy of surgery. Each case was classified by one infectious-disease specialist and one critical care specialist. Committee members did not examine cases from their own centers. All the work of the committee was completed in a blinded fashion before the treatment-allocation code was broken.

In the study, the term "sepsis" was defined by the inclusion criteria. This definition is similar to that proposed by Bone and colleagues.[13] Shock was defined as a systolic blood pressure of less than 90 mm Hg or the use of vasopressor drugs to maintain blood pressure. Gram-negative bacteremia was defined as the isolation of pathogenic gram-negative bacteria from at least one blood culture. We defined the adult respiratory distress syndrome using a modification of the criteria of Murray et al.,[14] requiring a total score of more than 7.5 based on points assigned for chest radiographic findings, amount of positive end-expiratory pressure required, and degree of hypoxemia. Renal failure was defined as a serum creatinine level of 177 μmol per liter (2 mg per deciliter) or more, or the need for dialysis. Hepatic failure was defined as the presence of two of the following: a total bilirubin level higher than 43 μmol per liter (2.5 mg per deciliter); aspartate aminotransferase or alanine aminotransferase levels more than twice the normal laboratory value; and a prothrombin time at least 1.5 times the normal value or a partial-thromboplastin time at least 1.2 times the normal value. Renal and hepatic failure were defined as acute if they had not been present before the development of sepsis. Disseminated intravascular coagulation was defined as both a platelet count of less than 100,000 cells per cubic millimeter (or a decrease of more than 50 percent from a previously measured value) and a prothrombin time at least 1.5 times the normal value or a partial-thromboplastin time at least 1.2 times the normal value. If coagulation abnormalities qualified a case as one of disseminated intravascular coagulation, they were not used for the classification of hepatic failure.

Antibiotic treatment was considered adequate if within one day of infusion of the study material the patient received an antibiotic to which each isolated organism was sensitive. Patients with pseudomonas infection of the respiratory tract needed either two classes of antibiotics or imipenem or a third-generation cephalosporin to which the organism was susceptible. Patients with ruptured bowel needed appropriate antibiotic coverage for enteric facultative and anaerobic gram-negative bacteria, including *Bacteroides fragilis*.

Statistical Analysis

The sample size was calculated to detect a 50 percent reduction in mortality among patients with gram-negative bacteremia at day 14, the midpoint between treatment and the end of follow-up. For the calculation we assumed a mortality of 30 percent in the placebo group, an expected incidence of gram-negative bacteremia of 40 percent in patients with sepsis, an alpha error of 0.05, and a beta error of 0.2.

For the trial we used the Harrington modification of the O'Brien–Fleming group sequential boundaries,[15] with two interim analyses conducted by the coordinating center. The results of each analysis were provided to the independent Safety and Efficacy Monitoring Committee. All others were blinded to these interim results.

Groups were compared by Student's t-test or the Wilcoxon rank-sum test for continuous variables and chi-square tests for categorical variables. All tests of significance were two-tailed. To analyze the difference in mortality over the 28-day period after therapy, Kaplan–Meier survival curves were constructed for the two study groups and compared by the Wilcoxon statistic. In a further analysis, patients were stratified according to each strong prognostic factor, and the Cochran–Mantel–Haenszel statistic was used to test whether the treatment effect of HA-1A remained significant after adjustment for the distribution of prognostic factors in the two study groups. A Cox proportional-hazards model was used to control for the influence of pretreatment shock and the APACHE II score on the treatment effect of HA-1A. Because a group sequential method of analysis was used, a P value of less than 0.049 was considered significant.

Results

There were 543 patients (262 HA-1A recipients and 281 placebo recipients) in the trial, of whom 200 (37 percent) proved to have had gram-negative bacteremia according to cultures of blood drawn before enrollment. The analysis of efficacy of HA-1A in this report is based on these 200 patients with gram-negative bacteremia. Information was available on all of them through day 14 and on 197 (99 percent) through day 28. The three patients who were lost to

follow-up, all in the placebo group, were discharged from the hospital.

Comparisons between Study Groups

Among the patients with gram-negative bacteremia, 105 received HA-1A and 95 received placebo. The treatment and placebo groups were well balanced with respect to demographic characteristics and underlying diseases (Table 1), the distribution of anatomical sources of bacteremia (Table 2), and causative organisms (Table 3). As shown in Table 4, the patients with gram-negative bacteremia were severely ill when they entered the study. The median intervals between the diagnosis of sepsis and infusion of the study drug were 9.3 hours in the placebo group and 14.3 hours in the HA-1A group. The temporal relation between the diagnosis of sepsis and the administration of adequate antibiotics was similar in the two groups ($P = 0.43$). Antibiotic treatment was judged to be adequate in 87 percent of the placebo recipients and 93 percent of those who received HA-1A. More than one adequate antibiotic was administered to 63 of the 95 placebo recipients (66 percent) and to 56 of the 105 recipients of HA-1A (53 percent). These differences between study groups were not significant. The use of corticosteroids, nonsteroidal antiinflammatory drugs, and narcotic antagonists was also well balanced (27 and 26 percent, 5 and 6 percent, and 6 and 5 percent, respectively). Surgical therapy for infection was judged adequate in 96 percent of those in the placebo group and 94 percent of those in the HA-1A group.

Efficacy of HA-1A in Gram-Negative Bacteremia

HA-1A significantly reduced mortality by 39 percent in the 200 patients with sepsis and gram-negative bacteremia. By 28 days after infusion there were 45 deaths among the 92 placebo recipients still being followed (49 percent) and 32 deaths among the 105 recipients of HA-1A (30 percent). The Kaplan–Meier survival curves for this population (Fig. 1) show that the reduction in mortality was evident as early as the first day after treatment, was sustained throughout the entire 28-day period of evaluation, and was significant (P = 0.014).

When mortality was examined in the patients with gram-negative bacteremia who were in shock before the infusion, a 42 percent reduction was observed. The number of deaths after 28 days was 27 of 47 patients (57 percent) in the placebo group and 18 of 54 patients (33 percent) in the HA-1A group ($P = 0.017$). The Kaplan–Meier survival curves for this population are shown in Figure 2. In the patients who were not in shock before infusion, the number of deaths by day 28 was 18 of 45 (40 percent) in the placebo group and 14 of 51 (27 percent) in the HA-1A group, a 33 percent reduction. A Cox proportional-hazards model was fitted to the survival data with treatment and shock as independent variables. This analysis indicated that shock was an important determinant of survival ($P = 0.047$) and that HA-1A reduced mortality in both patients with shock and patients without shock ($P = 0.012$).

Pretreatment APACHE II scores were highly correlated with death among the patients given placebo in all populations examined ($P = 0.0001$). In order to determine whether severely ill and less severely ill patients benefited from HA-1A, the patients with gram-negative bacteremia were divided into two groups according to whether their APACHE II scores were >25 or ≤25, the median value for the population. We fitted a Cox proportional-hazards model using treatment group and APACHE II score as the independent variables (Fig. 3). This analysis showed a significant reduction in mortality among both severely ill and less severely ill patients who received HA-1A ($P = 0.017$).

To examine more fully the independence of the treatment effect of HA-1A in gram-negative bacteremia, we used the Cochran–Mantel–Haenszel statistic for the following factors that might influence mortality: age, site of infection, category of underlying disease, neutropenia, pretreatment platelet count <100,000 per cubic millimeter, pretreatment arterial hypoxemia (ratio of partial pressure of arterial oxygen

Table 1. Demographic Characteristics of the Patients with Gram-Negative Bacteremia.*

Characteristic	Study Group	
	Placebo (N = 95)	HA-1A (N = 105)
Age (yr)	62.3±15.1	58.0±17.7
Sex (% male)	58	59
Weight (kg)	75.6±18.8	70.3±17.6
Race (% white)	73	62
Underlying diseases (%)		
Neoplasm		
Hematologic	8	9
Nonhematologic	20	26
Neutropenia	4	5
Diabetes mellitus	20	16
Chronic renal disease	7	10
Chronic liver disease	13	9
Alcoholism	17	10
Recent surgery	34	29
Recent trauma	3	7

*Differences between study groups were not significant. Plus–minus values are means ±SD.

Table 2. Sources of Gram-Negative Bacteremia.*

Source	Study Group	
	Placebo (N = 95)	HA-1A (N = 105)
	percent of patients	
Urinary tract	34	32
Intraabdominal site	22	25
Respiratory tract	12	12
Skin or wound	4	3
Foreign body	2	1
Other site	2	0
Multiple possible sources	12	7
Unknown	13	20

*Differences between study groups were not significant.

to fraction of inspired oxygen, <250), pseudomonas infection, adequacy of antibiotic therapy, and adequacy of surgery for infection. These variables have previously been associated with mortality in gram-negative bacteremia or were shown in this trial to be related to a fatal outcome in the placebo group. The treatment effect of HA-1A in patients with gram-negative bacteremia remained significant after we adjusted for each of these variables.

One or more of the major complications of sepsis — shock, disseminated intravascular coagulation, acute renal failure, acute hepatic failure, or the adult respiratory distress syndrome — were present at the time of infusion in 123 of the 200 patients with gram-negative bacteremia. During the first seven days after treatment, all evidence of these complicating conditions resolved in 26 of 62 patients given placebo (42 percent) and in 38 of 61 patients given HA-1A (62 percent; P = 0.024).

Table 3. Blood Isolates in Patients with Gram-Negative Bacteremia.*

Isolate	Study Group	
	Placebo (N = 95)	HA-1A (N = 105)
	number of patients	
Escherichia coli	42	45
Klebsiella	17	23
Enterobacter	7	10
Pseudomonas	16	9
Bacteroides	4	10
Proteus	4	10
Other gram-negative	11	21
Staphylococcus aureus	1	2
Enterococcus	1	4
Other gram-positive	7	0
Yeast	2	1

*Mixed bacteremia occurred in 12 of 95 patients (13 percent) in the placebo group and in 23 of 105 (22 percent) in the HA-1A group. Differences between study groups were not significant.

In order to evaluate the effectiveness of HA-1A further, the hospital records of all the patients who remained hospitalized 28 days after infusion were reviewed to determine survival status at discharge. At the time of the record review, 198 of the 200 patients had died or been discharged alive. Hospital records were available for 196 of these 198 patients (99 percent). The number of patients discharged alive was 45 of 93 (48 percent) in the placebo group and 65 of 103 (63 percent) in the HA-1A group (P = 0.038).

We analyzed the groups of patients who did not have gram-negative bacteremia to determine the specificity of protection by HA-1A. There was no significant difference in mortality between study groups among the 201 patients with nonbacteremic gram-negative infection (P = 0.30), the 51 patients with gram-positive infection without gram-negative infection (P = 0.15), the 7 patients with fungal infection

Table 4. Severity of Sepsis at Entry in the Patients with Gram-Negative Bacteremia.*

Variable	Study Group	
	Placebo (N = 95)	HA-1A (N = 105)
APACHE II Score	25.7±8.1	23.6±9.0
	percent of patients	
Hypotension, use of vasopressors, or both	51	51
Endotracheal intubation	55	54
Disseminated intravascular coagulation	21	18
Adult respiratory distress syndrome	13	9
Acute hepatic failure	26	19
Acute renal failure	46	35

*Differences between study groups were not significant. Plus–minus values are means ±SD.

(P = 0.14 by Fisher's exact test), the 84 patients with no infection identified (P = 0.96), or the entire population of 343 patients without gram-negative bacteremia (P = 0.68). When the results in patients without gram-negative bacteremia were combined with the results in those with the condition, the 28-day mortality rate in all the patients who received infusions (excluding 5 placebo recipients and 7 recipients of HA-1A lost to follow-up after discharge between day 3 and day 28) was 118 of 276 (43 percent) among placebo recipients and 100 of 255 (39 percent) among recipients of HA-1A (P = 0.24).

Safety

The incidence of adverse events, including serial changes in vital signs and laboratory measurements, was not significantly different in the HA-1A and placebo groups. There were no serious adverse reactions

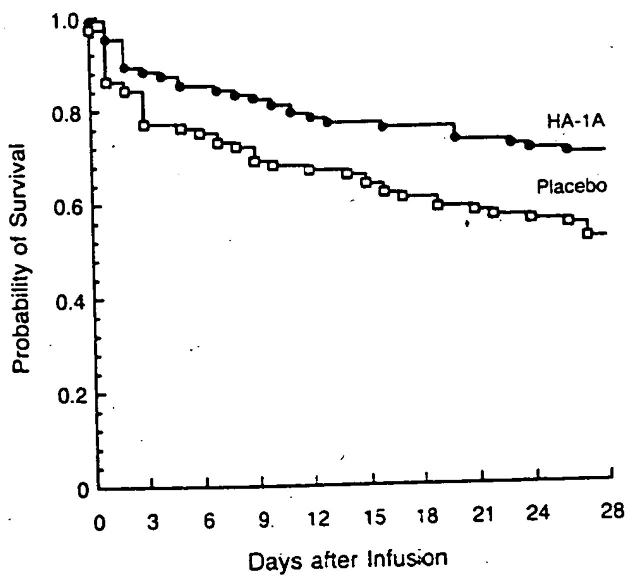

Figure 1. Probability of Survival in Patients with Gram-Negative Bacteremia.

Comparison of the cumulative survival estimates over a 28-day period for patients who received HA-1A (n = 105) and those who received placebo (n = 95) showed a 39 percent reduction in mortality with HA-1A treatment (P = 0.014).

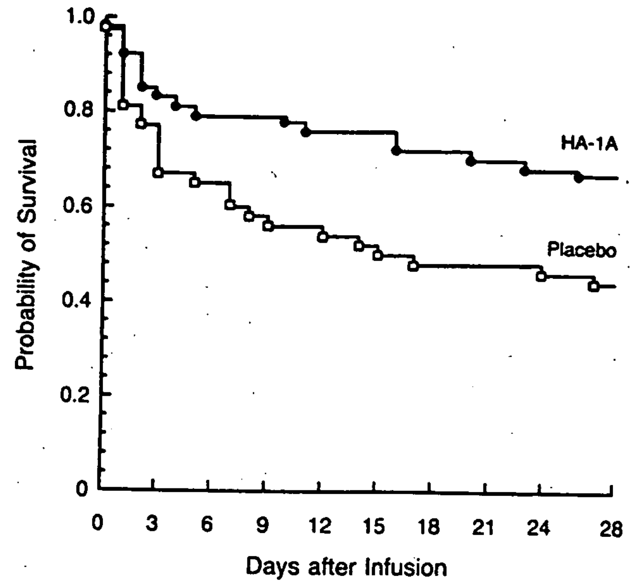

Figure 2. Probability of Survival in Patients with Gram-Negative Bacteremia and Shock at Entry.

Comparison of the cumulative survival estimates for patients who received HA-1A (n = 54) and those who received placebo (n = 48) showed a 42 percent reduction in mortality with HA-1A treatment (P = 0.017).

in the 291 patients given HA-1A. One patient had a transient episode of localized hives near the site of HA-1A infusion 10 to 15 minutes after the infusion, and it resolved without therapy. Near the end of the infusion, another HA-1A recipient had facial flushing and mild hypotension, which resolved without therapy in 30 minutes. Serum samples for assay of human antibody directed against HA-1A were obtained from 252 patients, 116 of whom had been given HA-1A. No patient had detectable anti–HA-1A antibodies.

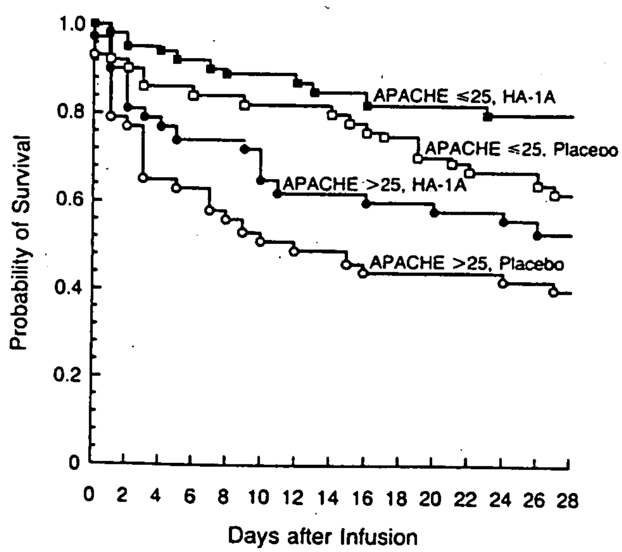

Figure 3. Probability of Survival in Patients with Gram-Negative Bacteremia Stratified According to APACHE II Score.

Patients were stratified into two groups according to whether their severity of illness as defined by th APACHE II score at enrollment was >25 (43 patients in the HA-1A group and 43 in the placebo group) or ≤25 (62 in the HA-1A group and 52 in the placebo group), the median score for the population. Analysis in a Cox proportional-hazards model showed a reduction in mortality with HA-1A treatment in both severely ill and less severely ill patients (P = 0.017).

Discussion

The results of this clinical trial show that adjunctive therapy with HA-1A, a human monoclonal antibody against endotoxin, reduces mortality significantly in patients with sepsis and gram-negative bacteremia. The reduction in mortality was apparent as early as day 1 after treatment and was sustained throughout the 28 days of observation. The treatment effect of HA-1A was strong and remained significant after adjustment for factors usually associated with a poor outcome. Both patients with a poor prognosis and those with a better prognosis, as defined by either the APACHE II score or the presence or absence of shock at the time of treatment, benefited from HA-1A therapy. HA-1A treatment was also associated with more rapid resolution of the major complications of sepsis than was placebo, and with a significantly higher rate of survival at hospital discharge.

The results of this clinical trial are similar to the results of a previous clinical trial of human polyclonal J5 antiserum, in which mortality in patients with gram-negative bacteremia was reduced by 37 percent and in patients with septic shock by 39 percent.[7] Comparable reductions of 39 and 42 percent were observed with HA-1A in the present trial. These two studies provide convincing evidence that immunotherapy with human antibody directed against a determinant expressed by the J5 mutant of *E. coli* confers substantial therapeutic benefit in patients with gram-negative bacteremia, including those with septic shock.

A concurrent control group was essential in this trial, since the mortality rate in gram-negative bacteremia is variable.[3] We decided that the trial of most value would be one that determined whether adding HA-1A to conventional therapy would improve survival in patients with sepsis and a presumptive diagnosis of gram-negative bacteremia. Human serum albumin was used because it is a safe, commercially available inactive control for the albumin used as an excipient in the HA-1A preparation. Other possible controls, such as human IgM, J5 polyclonal antiserum, and unrelated monoclonal antibodies, are experimental drugs, and their use would not have allowed extrapolation of the results to clinical practice.

Polyclonal J5 antiserum contains antibodies to both strain-specific and shared determinants of endotoxin from the J5 mutant. The shared determinants include lipid A, which is the most likely region to stimulate cross-reactive antibodies, because its structure is so highly conserved among gram-negative bacterial species.[16] Another bacterial mutant, the *Salmonella minnesota* Re strain, contains endotoxin consisting only of lipid A and one sugar. Human polyclonal IgM generated against this mutant protected animals from lethal

challenge with several different gram-negative bacteria.[17] The human monoclonal IgM of Teng et al. exhibits similar cross-reactivity with a wide variety of heterologous gram-negative bacteria and their endotoxins, including the J5 and Re mutants.[9] HA-1A, the antibody purified from this cell line by Centocor, behaves identically in vitro. Both the monoclonal supernatant fluid of Teng et al. and HA-1A protect rabbits with established pseudomonas bacteremia against death in a model in which J5 polyclonal antiserum was also shown to be effective.[10] HA-1A binds to lipid A and its analogues in enzyme-linked immunosorbent assays and in thin-layer chromatography, and the binding can be inhibited by the previous incubation of HA-1A with lipid A, monophosphoryl lipid A, polymyxin B, or a well-characterized murine IgG1 against lipid A, but not by incubation with an irrelevant IgG1[9,18] (and Ziegler EJ: unpublished data). Taken together, these data suggest that cross-protective IgM antiendotoxin antibodies are directed against an epitope on lipid A.

Some investigators have reported negative results with antiendotoxin core antiserum. Most of their studies were performed in mice. Although the reasons for discrepant results are not fully understood, several factors may contribute.[17,19] These include the relative resistance of rodents to endotoxin, the need to compromise host defenses severely in order to establish satisfactory animal models of gram-negative infection and endotoxemia, and the rather low affinity of cross-reactive antiendotoxin core antibodies as compared with type-specific antibodies. Recently, Baumgartner et al.[20] reported a lack of protection by a human monoclonal antibody derived from cells isolated from the same original clone as HA-1A, but the antibody was not produced or purified by the laboratory that produced HA-1A. Whether the discrepancies between their results and ours are due to differences in the monoclonal-antibody preparations or to the factors discussed above is not known. When species resembling humans in endotoxin sensitivity are studied, protection from J5 antibody can be demonstrated.[21] The negative results of Calandra et al. with J5 immunoglobulin in patients with gram-negative septic shock[22] may have been due to the absence of IgM in their preparation.

HA-1A has been administered in phase I trials to 15 patients with cancer[23] and in unblinded fashion to 34 patients with sepsis,[24] as well as to the 291 patients who received it in the present trial. In all these patients HA-1A was safe, well tolerated, and nonimmunogenic.

The treatment effect of HA-1A in this trial was specific for patients with sepsis and gram-negative bacteremia. Patients with gram-negative bacteremia frequently have endotoxemia.[5] Since HA-1A is an IgM antibody to endotoxin, it should be particularly effective against endotoxin in the bloodstream. The specificity of the HA-1A treatment effect for patients with gram-negative bacteremia supports the hypothesis that HA-1A protects patients by blocking the toxic effects of circulating endotoxin, which include induction and release of the mediators of shock and tissue damage. The pathogenesis of the sepsis syndrome in patients without gram-negative bacteremia presumably involves the release of mediators of sepsis through mechanisms that do not require endotoxin or by pathways in which endotoxin participates outside the bloodstream, in locations inaccessible to circulating HA-1A.

Sepsis requires prompt treatment, since the patient's condition often deteriorates rapidly, before the results of blood cultures are known. Therefore, in view of the lifesaving benefit of HA-1A in gram-negative bacteremia and the minimal risk associated with the administration of the antibody, empirical immunotherapy with HA-1A should be considered when patients with suspected gram-negative infection present with sepsis. The patients enrolled in this trial were severely ill and met specific criteria for the diagnosis of gram-negative sepsis. These criteria appear to identify patients with a 30 to 40 percent probability of having gram-negative bacteremia. The results of this trial should not be extended to infected patients who are not sick enough to meet our criteria for sepsis but should be applied only to patients with sepsis and presumed gram-negative bacteremia. Larger trials will be needed to determine whether HA-1A benefits patients with sepsis and focal gram-negative infections without bacteremia. Our results indicate that HA-1A is safe and that it substantially reduces mortality in patients with sepsis and gram-negative bacteremia. These findings illustrate the potential of human monoclonal immunotherapy in clinical medicine.

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MONOCLONAL ANTIBODIES AND THE TREATMENT OF GRAM-NEGATIVE BACTEREMIA AND SHOCK

IT has been 40 years since the first description of bacteremia due to gram-negative rods appeared.[1] It has always been assumed that the endotoxins (lipopolysaccharides) of gram-negative bacteria were responsible for the clinical manifestations of infections caused by these bacteria. This view was based on the observation that many of the characteristics of the infections could be reproduced by the infusion of endotoxin in laboratory animals and humans.[2] Since the 1960s, it has been well known that the seriousness of the underlying condition was one of the most important predictors of outcome for patients with gram-negative bacteremia. Furthermore, it is clear that patients with gram-negative bacteremia without shock have a much better prognosis than those in whom hypotension develops.

In the past 40 years, major advances in the treatment of gram-negative infections have been achieved, such as the development of powerful antimicrobial agents. In addition, important strides have been made in the treatment of many of the diseases that predispose patients to gram-negative bacterial infections, such as diabetes mellitus, certain neoplastic diseases, and burns. The development of critical care units and the new discipline of critical care medicine has led to improvement in the care of such patients. Despite these and other advances, however, cases of gram-negative bacteremia, with or without shock, continue to be numerous (approximately 100,000 to 300,000 per year), and the resulting deaths (30,000 to 100,000 per year) unacceptably so.

Bacterial endotoxins are composed of O-polysaccharide side chains (which are responsible for their O antigenicity), the R core that is shared among these bacteria, and lipid A, which is responsible for the biologic effects. When certain rough mutants of gram-negative bacteria that lack some of the polysaccharide side chains were recognized, studies were undertaken to determine whether primary immunization with

these mutants or the passive transfer of antiserum to them could protect animals against infection with heterologous smooth organisms. These experiments were successful.[3,4]

Eight years ago it was reported in the *Journal*[5] that the use of a polyclonal antiserum to an *Escherichia coli* rough mutant (J5) could reduce mortality in patients with gram-negative bacteremia, with or without hypotension. Although there was a significant reduction in mortality, a large number of patients still died. Subsequent studies showed that when the polyclonal antiserum to the J5 mutant was administered prophylactically to surgical patients at high risk for gram-negative bacterial infection, the rate of infection did not change, but the outcome in infected patients improved.[6] Other studies using an IgG antibody to the J5 mutant did not show any improvement in mortality rates in patients with gram-negative bacteremic shock.[7]

In an editorial that accompanied the earlier report in the *Journal*,[8] I asked, "Will modern monoclonal-antibody techniques make it possible to produce enough material to confirm and enlarge on the present studies?" The paper by Ziegler and her colleagues in this issue of the *Journal* provides an affirmative answer to my question.[9] In a multicenter trial involving more than 500 patients (200 of whom had gram-negative bacteremia), the authors report that mortality among patients with gram-negative bacteremia (with or without shock) who received a monoclonal IgM antibody (HA-1A) against the J5 mutant of *E. coli* was reduced as compared with mortality among controls who received serum albumin. Unfortunately, a large number of patients still died despite antibody therapy. Since previous studies have shown that most protective antibodies against endotoxins are IgM, I would have preferred that an IgM rather than serum albumin be used as placebo. As a matter of fact, I believe that rather than any placebo, the polyclonal anti-J5 antiserum should have been used. Such a comparison might have saved lives in the control group. I recognize that one might not want to compare one experimental agent with another, but in these circumstances I believe a strong argument could have been made for such a study. Given that the HA-1A antibody has a relatively short half-life (mean ±SEM, 15.9±1.5 hours),[10] it would be of interest to study patients who receive a number of doses of the antibody, rather than the single dose employed by Ziegler et al.[9] Of additional interest would be studies of primary immunization with the J5 antigen in patients who are at risk for gram-negative bacterial infections. Such patients include burn victims, those undergoing elective intraabdominal surgery, and — particularly pertinent as this editorial is being written — military personnel (Gelfand JA: personal communication).

Additional investigations to confirm the results reported in this issue of the *Journal* are required for many reasons, not the least of which are the findings of another recently reported study using a murine-derived but "humanized" IgM monoclonal antibody to the J5 mutant.[11] In a study very similar to Ziegler's,[9] the results were only partly confirmatory, since mortality was reduced in patients with gram-negative bacteremia but was not affected in patients with shock. In addition, recent studies in mice[12] failed to demonstrate protection by an anti-J5 monoclonal antibody when the animals were experimentally infected with gram-negative bacteria. Furthermore, the outcome for the patients treated with the monoclonal antibody[9] was not very different from the results when the polyclonal antiserum was used.[5] Although the monoclonal antibody has some advantages, such as the availability of large amounts, optimal therapy of this condition in the future will require additional agents.

Studies of the role of cytokines, in particular tumor necrosis factor and interleukin-1, in the pathogenesis of bacterial shock offer potentially exciting avenues for future clinical trials. Interleukin-1 and tumor necrosis factor are increased in the circulation of animals and humans who have received endotoxin or have septic shock.[13] Furthermore, the infusion of tumor necrosis factor or interleukin-1 induces in animals and humans hypotension and shock that are indistinguishable from those induced by endotoxin. Since these cytokines are important mediators of bacterial shock, would it not make sense to try to block them? Not only are antibodies to both cytokines available, but other potentially useful materials are also under study. They include soluble receptors for tumor necrosis factor,[14] which could bind the cytokine in vivo, thus inactivating it. In addition, an interleukin-1–receptor antagonist has been cloned, expressed, and shown to inhibit some of the biologic activities of interleukin-1 in animals and, more important, to prevent endotoxin and *E. coli*–induced shock in rabbits.[15,16] Thus, it is conceivable that in the future the treatment of patients with gram-negative bacteremia will include antibiotics directed at the bacteria, antibodies against the bacterial endotoxins, and agents that block or interfere with the mediators (cytokines) responsible for many of the adverse events that result in hypotension and its associated high mortality rate.

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